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Evaluation of Insecticidal Properties of *Datura innoxia* Seed Extract Against *Musca domestica* larva (*Diptera*)

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Abstract
This study investigates the insecticidal properties of *Datura innoxia* seed extract prepared using Soxhlet's method of extraction against *Musca domestica* larvae. Phytochemical analysis of *Datura innoxia* seed extract carried out by FTIR. The extract exhibited significant larvicidal activity, causing dose-dependent mortality in *M. domestica* larvae. The results suggest that *Datura innoxia* seed extract has potential as a natural insecticide for controlling *Musca domestica* populations, warranting further research on its efficacy and safety.

Keywords: *Musca domestica*, *Datura innoxia*, FTIR Insecticidal property, Phytochemical

Introduction
Musca domestica, the common housefly, is a significant pest species that plays a crucial role in spreading diseases such as typhoid, cholera, and dysentery to humans and animals (Wanaratana *et al*., 2013) [16]. The increasing resistance of *M. domestica* to synthetic insecticides has led to a growing concern about the environmental and health impacts of chemical pest control methods (Macovei *et al*., 2008) [8]. In recent years, there has been a shift towards exploring eco-friendly alternatives, including plant-based insecticides. *Datura innoxia*, a medicinal plant, has been traditionally used for its therapeutic properties and has been reported to possess insecticidal activities. (EI Zayyat *et al*., 2015). The present study aims to evaluate the insecticidal properties of *Datura innoxia* seed extract against *Musca domestica* larvae, with a focus on its potential as a natural and sustainable pest control agent. This study may provide valuable insights into the development of novel, environmentally friendly pest management strategies.

Material and Methods
1. Plant Sample
Plant samples were taken from Nashik region, Maharashtra, India. Agharkar Research Institute (ARI), Pune, identified and verified the collected aerial parts in the herbarium (Fig. 1). Collected fruits (Fig.2a, b, c&d) rinsed well in tap water and dissected to collect seeds. Seeds cleaned thoroughly in tap water and allow drying in shade for one week. Dried seeds grinded to coarsely powdered form in an electric grinder and stored in clean, dry and sterile jar (Khushboo Bharadwaj *et al*., 2016).

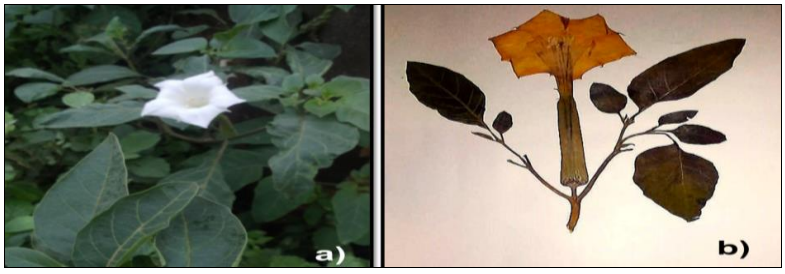

Fig 1: a) *Datura innoxia* Plant (Flowering Branch), b) Herbarium of *Datura innoxia* Plant

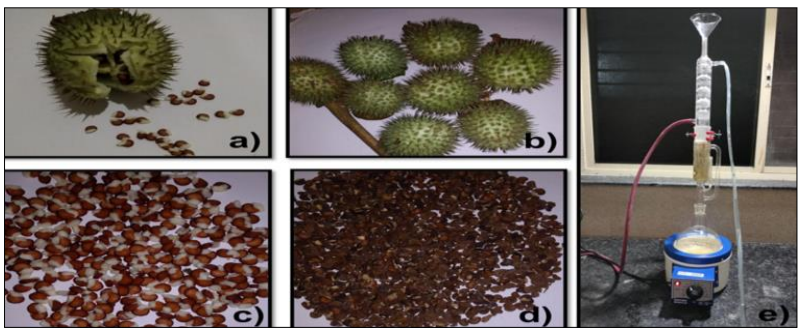

Fig 2: a) & b) *Datura innoxia* Fruits c) *Datura innoxia* Seeds freshly collected, d) *Datura innoxia* Seeds Shade dried e) Working Setup of Soxhlet Extraction

2. Datura innoxia Extraction Seed

50 g of Coarse powder of Datura innoxia packed in thimble of Whatman filter paper no. 1 and placed in Soxhlet extractor (Fig.2e). 500 cc of pure Methanol 60–80°C was employed as a solvent for the extraction, which was done at 60°C for eight hours.
To obtain enough extract, the extraction process was done five times. Solvent of final extraction was evaporated in rotary evaporator equipment (James, 2014).

3. FT-IR Analysis of Datura innoxia Seed extract

Datura innoxia seed extract was exposed to FTIR (Fourier Transformed Infrared Spectroscopy) analysis under IR range of 400-4000 cm-1 and related functional group were resolved (Coates, 2000), (Nandiyanto *et al*., 2019) [11]. Chemical identity and functional groups of plant extractions can be effectively identified by FTIR (Eberhardt *et al*., 2007) [1], (Shopska *et al*., 2013). FTIR Spectra of pure compound are frequently unique. Unknown functional group & plant chemicals can be specified by comparing spectrum of known molecules (Hazra *et al*., 2007).

4. Rearing of *Musca domestica*

Musca domestica were obtained from nearby area in Nashik region and reared in metallic rearing cage 50x50x50 cm (fig. 3a&b) in laboratory, provided a tiny cup of water and a cotton ball with 10% (w/v) milk and sugar as nourishment. At 28±3°C, 55–60% relative humidity, 12:12 light–dark, and covered with fine-mesh muslin cloth, separate cotton balls are kept with food for oviposition and raised for over 20 generations without coming into contact with pesticides (Jyoti C. *et al*., 2018).

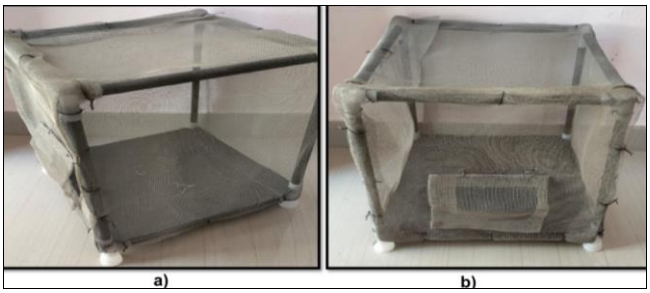

Fig 3: a) & b) Housefly (*Musca domestica*) Rearing Cage

Insecticidal activity of *Datura innoxia seed extract*

(a) (b) (c)

(d) (e) (f)

For the experiment, 4-5-day-old third-instar *Musca domestica* larvae from a group raised in a lab were utilized. Total six groups were prepared for experimentation; one group kept as a control group and other were treated. Each group comprise 10 larvae set in plastic jars supplied with food on cotton ball, tiny cup of water sufficient ventilation. To prevent escape, the jar was wrapped with muslin cloth and secured with a rubber band. Experiment carried out at 28±3°C and 55-60% relative humidity according to procedure of (Mallick *et al*., 2016) [10].
The mortality of *Musca domestica* larvae was recorded after every 24 hours, 48 hrs, 72 hrs and 96 hrs. For Negative Control Pyrethrin was employed as a positive control, while distilled water and petroleum ether (90:10) were used as a solvent without seed extract. Mortality of Targeted and non-targeted animals were reported for a time period of 24 hrs, 48 hrs, 72 hrs, and 96 hrs.
Mortality data used to determine LC50 using probit analysis. Microsoft Excel – 2007 was used for probit analysis, Regression Equation, R2 (Coefficient of determination) and Mean Percent Mortality, Standard Error and ANOVA analysis. Each set of experiment repeated thrice. Collected data was examined and determination of LC50 done by utilizing probit analysis (Finney,1971) [3].

Results

The percent mortality in this study increases in lower concentration however needed higher time exposure as

shown in table no. 1, Mortality rate of *M. domestica* larvae at lower concentration (08 µg/ml *D. innoxia* Seed Extract) shows mortality solely in 96 hrs time exposure which highest time exposure of the study, in 24 hrs, 48 hrs & 72 hrs time exposure not show mortality. While Highest mortality seen in every exposure time. As a result, *M. domestica* larval mortality increases with concentration and exposure duration.

As indicated in Table No. 1, the LC_{50} value of Datura innoxia seed extract for 24 hours, 48 hours, 72 hours, and 96 hours is 29.53 µg/ml, 23.12 µg/ml, 20.62 µg/ml, and 16.75 µg/ml, respectively. Higher concentration and longer exposure time period lead to lower lethal concentration as at lower concentration also lethality is higher for longer exposure.

Observation of lethal concentration of *Datura innoxia* seed extract against 3rd instar larva of *Musca domestica* are made that as time period of exposure increases lethality of extract increases even at lower concentration.

Phytochemical investigations of *Datura innoxia* extract fig. 5 reveals that presence of alkaloids like atropine, hyoscine, Phenolic compound such as flavonoids, Phenols and other compounds such as Triterpenes, Saponins, Essential oils and various antioxidants are responsible for lethality of *Musca domestica* at lower concentrations. *Musca domestica* for the first time, the larvicidal properties of *Datura innoxia* extract in petroleum ether as a solvent have been discovered, and the results of this study show that *Datura innoxia* seed extract possesses larvicidal potential against *Musca domestica*.

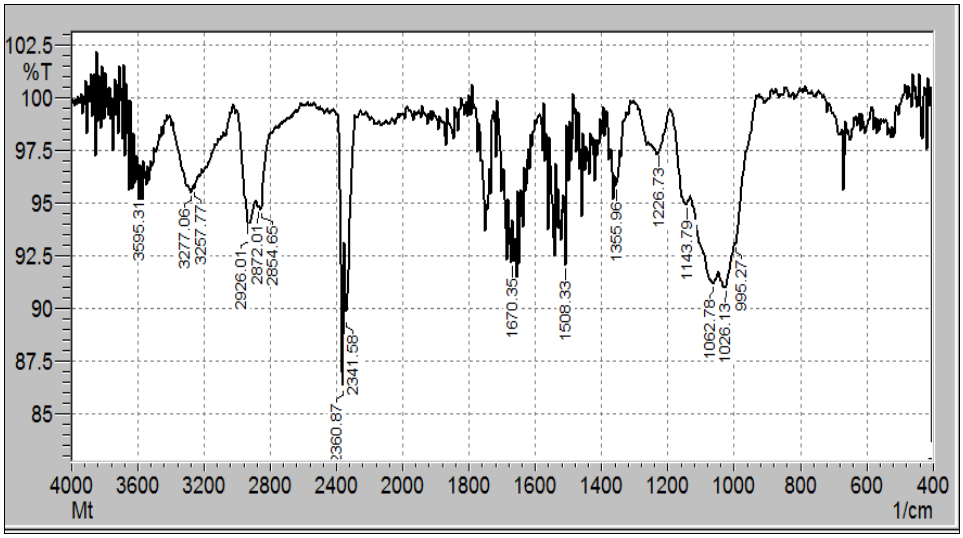

Fig 5: FT-IR Analysis of Datura Seed Extract

Discussion

Fourier Transform Infrared Spectroscopy Phytochemical investigation of *Datura innoxia* Seed Extract

A tiny film of the original material remains on the plate after the solvent has evaporated. The infrared spectra of the water-soluble and water-insoluble components of Datura Seed Extract (DSE) are shown in Fig. 5. Table No. 1 & 2 illustrates the absorption bands; their approximate locations and chemicals present. Oil-soluble compounds are extracted from plant material using petroleum ether, a popular non-polar solvent in herbal extraction. Non-polar substances like oils, fats, and other lipophilic chemicals are effectively removed by it (Subramani Parasuraman *et al*., 2014) [15].

The plants with the greatest pesticide potential were subjected to FTIR analysis in order to analyze the primary and secondary bioactive chemicals.

The existence of secondary metabolites, such as steroids, polyphenols, saponins groups, tannin, flavonoids, coumarins, carbohydrates, alkaloids, and terpenoids, in the ethanolic extract of Datura innoxia was confirmed by FTIR analysis, according to (Mahendra *et al*., 2017).The previously reported FTIR analysis is tied to the present D. innoxia IR spectrum data. The carbonyl (C=O) suggested the ester linkage, the alkyl group and aromatic ring, along with the phosphate group, were responsible for CH group stretching, and the extract exhibited notable peaks and potential chemical bonds that indicated the CH group was responsible for CH bending. Phytochemical research revealed the presence of anthraquinones, alkaloids, and flavonoids.

Table 1: Mortality of 3rd instar Larva *Musca domestica* treated with *Datura innoxia* Seed Extract at different concentration and time exposure

Concentration (µg/ml)	% Mortality in different exposure periods (% Mortality ±Standard error)			
	24	48	72	96
05	0	0	0	20±3.33
10	10±0.0	20±3.33	20±3.33	40±
15	20±3.33	60±5.62	60±5.62	70±5.62
20	40±3.33	70±5.62	70±5.62	80±5.62
25	70±5.62	80±5.76	80±5.76	90±5.77
30	100±0.0	100±0.0	100±0.0	100±0.0

Table 2: Shows log probit and regression studies on the lethality of *Datura innoxia* Seed extract against *Musca domestica* 3rd instar larva

Extract	Time of Exposure (hrs)	Regression equations $\{Y = y^- + b(x - x^-)\}$	R^2- values	LC_{50} Value (µg/ml)
Datura innoxia Seed	24	Y=5.7520X-3.63	0.6240	29.53
	48	Y=5.8983X-3.4523	0.7821	23.12
	72	Y=4.5390X-1.0740	0.7068	20.62
	96	Y=4.2697X-0.5297	0.6980	16.75

Lethality of Datura innoxia Seed extract against Musca domestica 3rd instar larvae

In the current study adulticidal activity of *Datura* Seed Extract toxicity calculated, Dose response relationship was observed, where higher concentration and longer exposure times led to increased mortality. On the negative control, no mortality was noted. *Datura* Seed Extract toxicity experiment demonstrated gradual decreasing LC50 for diverse exposure time Period, R2 values are in all records are almost closed to highly correlated dosage.

(Sharma P. *et al*., 2011) [13], determined toxicity of medicinal plant Artemisia *nilgirica and Annona squamosa, Blumea eriantha, Calotropis procera, Lavandula bipinnata* extract on *Musca domestica*, the population of *Musca domestica* can be lower by Artemisia *nilgirica and Annona squamosa* plant extract as they show significant biopesticidal activity against *Musca domestica*.

(Jawalkar N. *et al*., 2016) [12], focused on insecticidal activity of *Datura stramonium* seed extract against *Sitophilus oryzae* a storage pest. Storage pest can be control by using of *Datura stramonium* seed extract instead of chemical techniques and shown as a safer method for wheat grains.

Conclusion

Phytochemical investigations of *Datura innoxia* extract reveals that presence of alkaloids like atropine, hyoscine, Phenolic compound such as flavonoids, Phenols and other compounds such as Triterpenes, Saponins, Essential oils and various antioxidants are responsible for lethality of *Musca domestica* at lower concentrations. *Musca domestica* for the first time, the larvicidal properties of *Datura innoxia* extract in petroleum ether as a solvent have been discovered, and the results of this study show that *Datura innoxia* seed extract possesses insecticidal potential against *Musca domestica.*

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